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DEVELOPMENT OF SPECIAL BIOLOGICAL PRODUCTS (U)

Annual Progress Report

by

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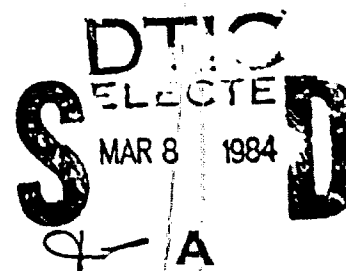
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19. Continued

Venezuelan Equine Encephalomyelitis (VEE)

Storage Stability

Chikungunya

HA Antigen

Eastern Equine Encephalitis (EEE)

Q Fever

Rocky Mountain Spotted Fever (RMSF)

20. ContinuedB. Tissue Culture

Two production lots of FRhL-2 and three of MRC-5 were stabilized and frozen this year. Two lots of FRhL-2 and partial lots of MRC-5 were shipped to USAMRIID. Microcarrier and suspension cultures were investigated. Aedes albopictus cells were tested.

C. VEE (C-84) Vaccine Development

The production scheme worked well and five lots of vaccine were prepared.

D. Storage Stability of Live, Attenuated TC-83 VEE Vaccine

Storage stability of TC-83, VEE vaccine was evaluated this year.

E. Chikungunya HA Antigen (Strain 168)

Chikungunya strain 168 was passed twice in suckling mice and brain tissue was extracted.

F. Venezuelan Equine Encephalomyelitis (VEE) HA Antigen (Trinidad Strain)

One batch of infected suckling mouse brains was harvested and the tissue processed in seven extractions.

G. Eastern Equine Encephalitis (EEE) HA Antigen (PE6 Strain)

A batch of infected suckling mouse brains was harvested, processed with seven extractions and pooled.

H. Rift Valley Fever (RVF) HA Antigen (Entebbe Strain)

The preparation of antigen with a titer of 1:1024 as requested was completed this year. A total of 77 extractions was made and 15 batches of infected suckling mouse livers harvested. Lot 1-80, 1800 vials, was shipped to USAMRIID. The remaining antigen is held in -20° storage, Lot 2-80 and at -70°C, Lot 3-80 frozen liquid. Two batches of antigen were made for use in preparing a hybridoma. Batch 37-80-3 (1:4096) was shipped to USAMRIID.

I. Eastern Equine Encephalitis (EEE) Vaccine Storage Stability Potency Testing

EEE vaccine Lot 1 (NDBR 104) was potency tested.

J. Q Fever Vaccine Storage Stability Potency Testing

Q fever vaccine (NDBR 105) was put on potency test.

K. Rocky Mountain Spotted Fever (RMSF) Vaccine

Potency tests performed on RMSF vaccine incorporating changes made by USAMRIID were again potency tested with protocols further modified. RMSF vaccine was sent to USAMRIID.

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## REPORT DOCUMENTATION PAGE

20. ABSTRACTL. Immunization of Employees

Plaque reduction neutralization tests were performed on sera from five employees receiving inactivated, VEE vaccine, C-84-1.



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### Summary

#### A. Rift Valley Fever (RVF) Vaccine Development

RVF vaccine production in FRhL-2 cell cultures was finished during this period with the completion of final container tests on Lot 20 Run 2, the labeling and storage at -20°C of Lot 15 Run 2 through Lot 20 Run 2 and the testing of Lots 15 through 20 for residual live virus.

The vaccine submission for Lots 11 through 20 was forwarded to USAMRIID.

Eight vials of RVF vaccine were shipped to USAMRIID.

The test for adventitious agents in Production Seed Virus (RVF 184 Ba) was completed in L-cells.

Potency testing of seven (7) lots of RVF vaccine was performed following protocols supplied by USAMRIID.

#### B. Tissue Culture

Two production lots of FRhL-2 and three of MRC-5 were stabilized and frozen this year. Two of the three MRC-5 lots and both FRhL-2 lots were certified. Two lots of FRhL-2 were shipped to USAMRIID along with partial lots of MRC-5.

Microcarrier and suspension cultures were investigated using MRC-5 and Vero cells. Aedes albopictus cells were tested for tumorigenicity and karyology. The automatic filler-sealer reached 85% efficiency this year.

#### C. VEE (C-84) Vaccine Development

The production scheme previously devised (Annual Progress Report-No. 44-95-0180-TSI002, January 1980, p. 27) worked well this year. Five lots of vaccine consisting of 304 liters were prepared. A total of 11,085 eggs were processed into 6,342 g tissue to prepare 2,195 rolling cultures of CEC. All testing has been satisfactory thus far.

#### D. Storage Stability of Live, Attenuated TC-83 VEE Vaccine

Storage stability of TC-83, VEE vaccine was evaluated this year.

#### E. Chikungunya HA Antigen (Strain 168)

Chikungunya Strain 168 was passed twice in suckling mice and brain tissue was extracted with sucrose-acetone. Six batches were extracted and Lot 1 of the series was shipped to USAMRIID.

F. Venezuelan Equine Encephalomyelitis (VEE) HA Antigen (Trinidad Strain)

One batch of infected suckling mouse brains was harvested when 10% mortality occurred and the tissue was processed in seven extractions. A pool at a titer of 1:2560 was prepared and frozen at -70°C.

G. Eastern Equine Encephalitis (EEE) HA Antigen (PE6 Strain)

One batch of infected suckling mouse brains was harvested and processed with seven extractions. A pool was made and frozen at -70°C.

H. Rift Valley Fever (RVF) HA Antigen (Entebbe Strain)

The preparation of antigen with a titer of 1:1024 as requested (Ref. SGRD-UIV-1 3/1/79 and 7/17/79) was completed this year.

A total of 77 extractions was made and 15 batches of infected suckling mouse livers were harvested. Lot 1-80, 1800 vials, was shipped to USAMRIID. The remaining antigen is held in -20°C storage, Lot 2-80 and at -70°C, Lot 3-80 frozen liquid.

Two batches of antigen were prepared without HSA present for use in preparing a hybridoma. Batch 37-80-3 (1:4096) was shipped to USAMRIID.

I. Eastern Equine Encephalitis (EEE) Vaccine Storage Stability Potency Testing

Two runs of EEE vaccine Lot 1 (NDBR 104) stored at -20°C for ten years were potency tested.

J. Q Fever Vaccine Storage Stability Potency Testing

Two lots of Q fever vaccine (NDBR 105) were put on potency test.

K. Rocky Mountain Spotted Fever (RMSF) Vaccine

Potency tests were performed on RMSF vaccine (MNLBR 108) Lots 1, 2 and 3 and USAMRIID Lot 1 incorporating changes made by USAMRIID.

The above four lots of RMSF vaccines were again potency tested with protocols further modified by USAMRIID.

RMSF vaccine from Lots 2 and 3 was sent to USAMRIID.

L. Immunization of Employees

Plaque reduction neutralization tests were performed on pre- and post vaccination sera from five employees receiving inactivated, VEE vaccine, C-84-1. Three subjects developed antibody titers greater than the required 1:80 and two did not.

Foreword

The authorization for the work contained herein was authorized under Contract No. DAMD17-78-C-8018, titled, "Development of Special Biological Products".

This annual report covers the period of January 1, 1980 through December 31, 1980. In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

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## Rift Valley Fever (RVF) Vaccine Development

### I. Introduction

RVF vaccine production in FRhL-2 cell cultures was finished during this period with the completion of final container tests on Lot 20 Run 2, the labeling and storage at -20°C of Lot 15 Run 2 through Lot 20 Run 2 and the testing of Lots 15 through 20 for residual live virus.

The vaccine submission for Lots 11 through 20 was forwarded to USAMRIID.

Eight vials of RVF vaccine were shipped to USAMRIID.

The test for adventitious agents in Production Seed Virus (RVF 184 Ba) was completed in L-cells.

Potency testing of seven (7) lots of RVF vaccine was performed following protocols supplied by USAMRIID.

### II. Vaccine Production

During 1980, Lot 20 Run 2 of RVF vaccine was completely tested with satisfactory results as per the vaccine submission. Final container tests are summarized in Table 1.

Lots 15 Run 2 through 20 Run 2 were labeled, packaged and placed at -20°C storage in the Bally freezer.

The quantities of the completed vaccines on hand which are stored at -20°C are recorded in the inventory section of this report.

The vaccine submission for Lots 11 through 20 was forwarded to USAMRIID.

### III. Residual Live Virus Testing

Testing for residual live virus (Annual Progress Report-No. 44-95-0180-TSI002, January 1980, pp. 2-3) was completed on RVF vaccine Lots 15 through 20 by the Vero cell culture-weanling mouse test with negative results.

### IV. Vaccine Shipments

Five (5) vials of Lot 1 Run 1 and 3 vials of Lot 1 Run 2 RVF vaccine (TSI-GSD 200) were shipped to USAMRIID.

#### V. Production Seed Testing for Adventitious Agents

Production Seed Virus (RVF 184 Ba) was tested for adventitious agents against three cell lines; L-cells, MRC-5 and Vero.

The passes have been completed in only L-cells. These data, included in a separate report to USAMRIID June 4, 1980 indicate the absence of live virus adventitious agents in L-cells.

Rabbit anti-RVF serum was produced during the last quarter of 1980 so attempts can be made to complete the testing for adventitious agents in Vero and MRC-5 cell lines.

#### VI. Potency Test Results

Potency testing was performed on six (6) lots of RVF vaccine, from virus propagated in FRhL-2 cells and one (1) lot of BHK-21 grown virus. The results in Table 2 reveal that none of the lots would have passed our minimum requirements of 0.02 ml or less deemed necessary to protect 50% of the immunized animals.

The best lot tested was the vaccine produced for veterinary use in BHK-21 cells. This vaccine with an ED<sub>50</sub> of 0.028 had 18 survivors out of 20 test mice when the vaccine was diluted 1:4. The only vaccine prepared in FRhL-2 cells that protected over 50% of the mice at 1:4 was Lot 7 Run 1 with 12 survivors from 20 test mice at the above dilution.

However it should be borne in mind that the results shown above were attained after administration of one dose of vaccine, whereas previous tests were conducted on the basis of a two dose regimen.

Table 1

Rift Valley Fever (RVF) Vaccine Development

RVF Vaccine (FRhL-2), Inactivated, Dried  
(TSI-GSD 200)

Final Container Tests-Lot 20 Run 2 (5 Dose)

Test	Result
Sterility	<u>1</u> S
General Safety	S
Formalin Content (%)	0.004
Moisture Content (%)	0.22
Potency (ED <sub>50</sub> /ml)	0.005*
Potency, Ref. Vaccine <sup>2</sup>	0.012

<sup>1</sup> S = Satisfactory<sup>2</sup> NDBR 103, GMK Vaccine.

\*Two-dose potency test.

Table 2

## Rift Valley Fever (RVF) Vaccine Development

## RVF Potency Testing\*

Vaccine	ED <sub>50</sub>	Survivors/Total Immunized @1:4 dilution of vaccine
Lot 1 Run 1	>0.05	2/20
Lot 4 Run 2	>0.05	5/20
Lot 5 Run 1	>0.05	8/20
Lot 6 Run 2	>0.05	5/20
Lot 7 Run 1	0.042	12/20
Lot 8 Run 2	>0.05	5/20
BHK-21 Lot 1	0.028	18/20

\*One dose potency test.

## Tissue Culture

### I. Introduction

Two production lots of FRhL-2 and three of MRC-5 were stabilized and frozen this year. Two of the three MRC-5 lots and both FRhL-2 lots were certified. Two lots of FRhL-2 were shipped to USAMRIID along with partial lots of MRC-5.

Microcarrier and suspension cultures were investigated using MRC-5 and Vero cells. Aedes albopictus cells were tested for tumorigenicity and karyology. The automatic filler-sealer reached 85% efficiency this year.

### II. Process Studies

#### A. Production Cells

##### 1. FRhL-2 Diploid Cell Line

Two production lots of FRhL-2 were prepared and certified as shown in Table 3. The chromosome analyses are shown in Table 4. Lot 21 (uncertified lot) was included for comparison. Polyploidy for Lot 20, reported by Metpath the previous year to be 12% was reexamined and found to be 3% in our hands.

Lots 20 and 25 were shipped to USAMRIID, depleting the supply of these lots. A total of 39 ampules of various lots was used for experimental and testing procedures.

##### 2. Primary Chick Embryo Cells

In addition to the 2,195 rolling cultures of CEC prepared for C-84 VEE vaccine production, several small batches were prepared for testing purposes.

##### 3. MRC-5 Diploid Cell Line

Three production lots of MRC-5 were prepared as shown in Table 5. Lots 9 and 10 were completely tested while a few tests remain to be completed on Lot 12. This lot had a higher number of cultures at harvest since a 1:4 split was used at P22 so that sufficient cells would be on hand to use the Cozzoli automatic filler-sealer. An 85% efficiency of sealing was accomplished with this run.

Chromosome analyses on these lots including Lot 5 for comparison were completed as summarized in Table 6. The high polyploidy for Lot 3 reported by Metpath last year as 15% was only 2% upon our examination.

#### 4. IMR-90 Diploid Cell Line

Four ampules were used for testing purposes.

#### 5. Others

No work was done with FCL-7, IMR-91, DEC or Dog Kidney cells.

### III. Experimental

#### A. Microcarrier

##### 1. Small Spinner Culture

Small cultures (100-800 ml) of MRC-5 were grown on Cytodex I. Results were not uniform in that the beads are not homogeneous in size. Smaller beads attract cells and sheet easily while many of the larger beads had no cells attached, even after multiple cell inoculums. These initial trials were at a level of 4-8 cells/bead inoculum. A later communication from Pharmacia indicated 10 cells/bead is a minimum starting point for MRC-5 cultures.

##### 2. Four-liter Fermentor

A New Brunswick 4-liter fermentor was modified by using a motor from a roller apparatus for low-speed mixing and using a silicone-clad stirrer. The design keeps the microcarrier beads from accumulating beneath the center shaft. Filtered, 5% CO<sub>2</sub>/air was used as an air overlay and discharge air was passed through two Pall 0.45  $\mu$  in-line filters terminating in a one liter bottle containing Clorox.

Three liters of Cytodex I/MRC-5 cells (P24) were prepared over an 11 day period -  $73 \times 10^6$  beads/ $1500 \times 10^6$  total cells inoculated in 4 bi-daily inoculums or 20 cells/bead. No cells were added the last three days but the stirring speed was increased from 30 rpm to 60 rpm. Approximately 85% of the beads were sheeted after 11 days. The remaining beads were either devoid of cells or had degenerating cells attached.

The culture was rinsed four times with Hanks BSS and twice with EMEM containing 2% (V/V) HSA-(MM). One liter of EEE/PE6 virus diluted  $10^{-5}$  in MM was adsorbed to the culture for 2 hours at 15-20 rpm. This was drawn off and 2½ liters fresh MM was added - 3 L total. The serum concentration was calculated to be less than one part/million. After 48 hours there was 80-90% CPE present and the culture was harvested, filtered and inactivated with Formalin (0.05%). The harvested material showed  $10^{9.5}$  TCD<sub>50</sub> per 0.5 ml and  $4.3 \times 10^8$  PFU per 0.1 ml in Vero cells. Post-filtration virus was  $10^9$  TCD<sub>50</sub> per 0.5 ml and  $2.1 \times 10^8$  PFU per 0.1 ml.

Cells from control beads (taken from fermentor prior to infection) were grown in monolayer and passed once prior to karyologic

analysis. The results are shown in Table 7. Included is a comparison of similar cells grown in monolayer and checked after 23 passages. As indicated, the aberration rate on the cells from the Cytodex fermentor was twice that normally experienced. Aberrations were mainly due to breaks (10% of the 14%).

Some of the items to be looked at are use of an earlier passage cell, higher initial inoculum of cells and other, perhaps more uniform microcarriers such as "Cytospheres" (Lux).

#### B. Suspension Culture

Passage 137 Vero cells were placed in suspension at a concentration of  $1.95 \times 10^5$  cells/ml. Cells were trypsinized four days consecutively, then five days consecutively after a two day period. After the second week, the concentration was  $3.9 \times 10^5$ /ml, but still some clumps. Trypsinization resumed after 2 days for three days, at which time the cell concentration was  $7.8 \times 10^5$ /ml. The culture was frozen down with 7% DMSO at this stage for the holiday period.

A second approach to adaptation has been started using Ca-free media for monolayer growth. These cells are currently in their 3rd passage.

#### C. Mycoplasma Testing (Fluorescence Microscopy)

DNA staining procedures using 4'-6-diamidino-2-phenylindole fluorochrome stain has been used on MRC-5 cells. Positive cultures have been prepared and stained. Parameters of the test such as stain concentration, time of staining, fixation and type of fixatives are being defined. This is part of the work being done for faster-screening of cultures and materials used in tissue culture.

#### D. Test Cells

##### 1. L929 Cells

The cell line was obtained from the ATCC at pass 556, processed to pass 559 and harvested. Preparation of ampules is summarized in Table 8.

##### 2. CV-1 Cells (African Green Monkey Kidney)

These cells were checked at P41 for Mycoplasma and diploid characteristics. They are free of Mycoplasma according to the standard 21 CFR 610.30 test and by fluorescence. The chromosome analysis is shown in Table 9. As indicated, they are no longer diploid as was the case at P20 (ATCC).

### 3. Aedes albopictus Cells (C6/36)

Passage 8 of this clone was obtained from USAMRIID and was passed to passage 11 for karyologic studies and tumorigenic potential. A separate report on these studies was transmitted to USAMRIID. No oncogenic tendencies were observed in the newborn hamster test and no overt signals were observed in the karyologic studies as being atypical for this cell line.

#### E. Serum

Samples of serum from two manufacturers were obtained and tested. One (Reheis) was rejected since it was contaminated. The other (Hyclone 100277) was as good as, if not slightly better than, our present lot in low concentration cell growth and maintenance (Skinny Test). It was sterile, free of PPLO and was procured.

#### F. R/S Freeze Drier

An automatic liquid nitrogen trap was installed and tested. Chronic leaks around the door gasket and drain line have delayed the use of this drier for experimental work.

### IV. Cell Inventory

A summary of the inventory and use of ampules for the year is in Table 10. As seen, a total of 1129 ampules were shipped to USAMRIID. Only 136 ampules were used here for assorted test and production uses. This was lower than last year, primarily due to the use of primary CEC cells for production of VEE vaccine.

### V. Conclusion

The certified cell system worked well this year. Microcarrier and suspension culture should take increased effort in this coming year.

Table 3

Certification of Two Lots of Male Fetal Rhesus Lung Diploid  
Cells - FRhL-2 P16

Item	Lot 24	Lot 25
No. bottles harvested (150 cm <sup>2</sup> )	720	720
Surface area (cm <sup>2</sup> )	108,000	108,000
Total cells (x 10 <sup>10</sup> )	1.53	1.30
Cells/cm <sup>2</sup> (x 10 <sup>5</sup> )	1.4	1.2
No. amps (plastic-heat sealed)	486	598
Cells/amp (x 10 <sup>6</sup> )	31.4	21.8
Viability (%)	90	96
Sheetability: 1 amp - 10 x 75 cm <sup>2</sup>	2 days	2 days
1 amp - 20 x 75 cm <sup>2</sup>	4 days	4 days
1 amp - 850 cm <sup>2</sup> roller	4 days	4 days
Bulk sterility	Sterile	Sterile
2 week hold of 1-2% cell sample after harvest	Normal	Normal
30 day hold of harvest fluids	Sterile	Sterile
PPLO: frozen/thawed cells	Negative	Negative
harvest growth fluids	Negative	Negative
2-week sheetability growth fluids	Negative	Negative
Hemadsorption - cells from sheetability	Negative	Negative
2 week hold-harvest cells	Negative	Negative
M. tuberculosis (Lowenstein-Jensen)	Negative	Negative
Tissue Culture safety a) RK-13	Passes	Passes
b) MRC-5	Passes	Passes
c) CV-1 + subpass	Passes	Passes
d) CEC	Passes	Passes
Egg safety (allantoic)	Passes	Passes
Tumorigenicity (newborn hamster/ALS)	Negative	Negative
Karyology	Passes	Passes

Table 4  
Chromosome Analysis on Three Lots of  
FRhL-2 (P17)

No. of Chromosomes	Lot 21	Lot 24	Lot 25
	(No. of cells)		
39	2	0	1
40	2	3	3
41	7	7	6
42	89	90	90
Polyploidy	1.0%	1.2%	1.6%
Aberration	5.0%	7.0%	8.0%
Total No. Cells Counted	100	100	100
Modal Chromosome No.	42	42	42

Table 5  
 Certification and Testing Status of Three Lots of Male  
 Fetal Human Lung Diploid Cells  
 MRC-5 P23

Item	Lot 9	Lot 10	Lot 12
No. bottles harvested	720	720	952 <sup>1</sup>
Surface area (cm <sup>2</sup> )	108,000	108,000	142,800
Total cells (x 10 <sup>10</sup> )	1.07	0.96	1.6
Cells/cm <sup>2</sup> (x 10 <sup>5</sup> )	0.9	0.9	0.1
No. amps (plastic-heat sealed)	550	408	765
Cells/amp (x 10 <sup>6</sup> )	21.3	23.6	20.6
Viability (%)	98	98	92
Sheetability: 1 amp - 10 x 75 cm <sup>2</sup>	3 days	2 days	3 days
1 amp - 20 x 75 cm <sup>2</sup>	4 days	3 days	3 days
1 amp - 850 cm <sup>2</sup> roller	4 days	3 days	5 days
Bulk sterility	Sterile	Sterile	Sterile
2 week hold of 1-2% cell sample after harvest	Normal	Normal	Normal
30 day hold of harvest fluids	Sterile	Sterile	Sterile
PPL0: frozen/thawed cells	Negative	Negative	ND <sup>2</sup>
harvest growth fluids	Negative	Negative	ND
2-week sheetability growth fluids	Negative	Negative	ND
Hemadsorption: cells from sheetability	Negative	Negative	Negative
2 week hold-harvest cells	Negative	Negative	Negative
M. tuberculosis (Lowenstein-Jensen)	Negative	Negative	Negative
Tissue culture safety a) RK-13	Passes	Passes	Passes
b) CV-1 + subpass	Passes	Passes	Passes
c) FRhL-2	Passes	Passes	Passes
d) CEC	Passes	Passes	ND
Egg safety (allantoic)	Passes	Passes	Passes
Tumorigenicity (newborn hamster/ALS)	Negative	Negative	Negative
Karyology	Normal	Normal	ND

<sup>1</sup> Passage 23 done with 1:4 split.

<sup>2</sup> ND = Not Done

Table 6

Chromosome Analysis on Three Lots of  
MRC-5 (P24)

No. of Chromosomes	Lot 5	Lot 9	Lot 10
	(No. of cells)		
44	3	1	0
45	12	6	7
46	85	93	92
47	0	0	1
Polyploidy	2.2%	1.8%	1.8%
Aberration	7.0%	5.0%	9.0%
Total No. Cells Counted	100	100	100
Modal Chromosome No.	46	46	46

Table 7

Chromosome Analysis on MRC-5 Cells Taken  
from 4-liter Fermentor  
(Cytodex I)

No. of Chromosomes	Cytodex I <sup>1</sup> Cells	Typical <sup>2</sup>	
		P24	MRC-5
44	6		1
45	12		9
46	82		90
47	0		0
Polyploidy	1.4%		1.9%
Aberration	14.0%		7 %

<sup>1</sup> Passed twice in monolayer - passage level 26 or more.

<sup>2</sup> Average of Lot 5, 9 and 10 MRC-5.

Table 8  
Preparation of L-929 Mouse Fibroblasts - P559

Item	Result
No. bottles harvested (150 cm <sup>2</sup> )	17
Surface area (cm <sup>2</sup> )	2550
Total cells (x 10 <sup>8</sup> )	4.86
Cells/cm <sup>2</sup> (x 10 <sup>5</sup> )	1.9
No. amps	90
Cells/amp (x 10 <sup>6</sup> ) (av. 2 amps)	5.1
Viability (%)	95
Sheetability: 1 amp - 2 x 75 cm <sup>2</sup>	4 days

Table 9

## KARYOLOGICAL ANALYSIS ON CV-1 CELLS (P41)

NO. OF CELLS COUNTED ..... 100

NO. OF CHROMOSOMES	56	60	61	62	63	64	65	66	68	69
--------------------	----	----	----	----	----	----	----	----	----	----

NO. OF CELLS	1	10	7	12	9	30	27	2	1	1
--------------	---	----	---	----	---	----	----	---	---	---

POLYPLOIDY	- 6.0%
TOTAL BREAKS	- 5
TOTAL GAPS	- 4
DICENTRICS	- 1
MARKER CHROMOSOMES	- 98

Hyperdiploidy was 89% while only 10% was diploidy. This indicates CV-1 (P41) is no longer diploid cell line. Polyploidy was 6% on the basis of 500-cell count, which is well beyond upper acceptable limit. Moreover, virtually almost all the cells observed (98%) had chromosome aberration(s), which included breaks, gaps, dicentric and stable marker chromosomes. Most of the marker chromosomes were giant-acrocentric. All of these data indicate that CV-1 cells (P41) under study is no longer normal line at least in view of karyologic analysis. Since not all mutations lead to carcinogenesis, this cytogenetical abnormality does not necessarily mean that CV-1 cells under study are malignant. However, checking the oncogenicity with this cell line in vivo may be necessary.

## Comparison - ATCC Analysis at P22

NO. OF CHROMOSOMES	56	57	58-60	61-	64-	98-	110-	120
--------------------	----	----	-------	-----	-----	-----	------	-----

NO. OF CELLS	1	1	8 41	1	1	1	1	1
--------------	---	---	------	---	---	---	---	---

Table 10

Cell Inventory and Use  
1980

Item #	Cell	Lot #	Pass	Date Frozen	Ampule Cell Count (x 10 <sup>6</sup> )	Viability (%)	No. Amps Jan. 80	Amps Shipped	Amps Used	Current Inventory	Use
1	FRhL-2	PS	10	2/14/78	6.4	94-98	84	-	2	82	Certified Cells for Vaccine Prep. →
		8-OPS	17	11/23/76	8.0	75	284	-	-	284	
		14	17	2/22/78	26.0	96-98	307	-	19	288	
		20	16	2/1/79	41.2	94	345	344	1	0	
		21**	16	3/20/79	29.8	93	300	-	3	297	
		24	16	4/2/80	31.4	90	-	-	7	449	
2	FCL-7	Seed	7	12/22/77 (rec'd.)	-	-	1	-	-	1	
		OPS	16	5/17/77	5.4	90-94	193	-	-	193	
3	IMR-90	MS	10	5/16/77	4.9	94	180	-	-	180	
		PS	14	6/1/77	3.4	99	46	-	-	46	
		1	21	11/14/77	37.7	94-97	309	-	4	305	
4	IMR-91	MS	10	8/31/78	5.2	100	98	-	-	98	

continued-----

Table 10  
Cell Inventory and Use  
1980

Item #	Cell	Lot #	Pass	Date Frozen	Ampule Cell Count (x 10 <sup>6</sup> )	Viability (%)	No. Amps Jan. 80	Amps Shipped	Amps Used	Current Inventory	Use
5	MRC-5	PS	17	6/7/77	7.0	100	29	-	5	24	Certified Cells for Vaccine Prep. →
		5	23	1/11/78	42.0	92-96	349	100	5	244	
		6**	23	8/6/79	33.0	93	44	10	12	12	
		7**	23	9/14/79	64.5	96	196	100	24	72	
		9	23	6/10/80	21.3	98	-	-	11	529	
		10	23	7/15/80	23.6	98	-	-	-	379	
		12	23	11/4/80	20.6	92	-	-	-	621	
6	DEC (Duck)	1	Primary	2/26/75	152.0	93	6	-	-	6	
7	DK (Dog Kidney)	Dow Chem.	Primary	4/5/77 (rec'd.)	-	-	1375	-	-	1375	
8	BSC-1	-	76	2/14/75	14.0	84-87	19	-	-	19	Test Cells →
9	CV-1	-	29	12/21/76	1.3	85	10	-	-	10	
			36	10/20/78	-	-	81	-	14	67	
10	KB	-	-	3/18/75	14.0	89-98	51	-	3	48	

Table 10

Cell Inventory and Use  
1980

Item #	Cell	Lot #	Pass	Date Frozen	Ampule Cell Count (x 10 <sup>6</sup> )	Viability (%)	No. Amps Jan. 80	Amps Shipped	Amps Used	Current Inventory	Use
11	LLC-MK2	-	264	2/11/75	4.0	78	32	-	-	32	Test Cells
12	RK13	-	73	6/16/75	9.0	83	39	-	-	37	
13	Vero	-	122	4/24/75	2.0	82	47	-	6	41	
		-	137	8/30/79	32.4	96	91	-	10	81	
14	BHK-21	-	57	5/14/79 (rec'd.)	-	-	5	-	-	5	
			58	6/12/79	30.0	98	21	-	-	21	
15	L-929	-	556	12/18/79 (rec'd.)	-	-	1	-	-	1	
			559	1/10/80	-	-	-	-	-	87	
16	A. albopictus (C6/36)	-	8	2/6/80 (rec'd.)	-	-	-	-	1	5	

\*PS = Production Seed; OPS = Old Production Seed; MS = Master Seed.

\*\*Shipped as uncertified cells.

VEE (C-84) Vaccine DevelopmentI. Introduction

The production scheme previously devised (Annual Progress Report-No. 44-95-0180-TSI002, January 1980, p. 27) worked well this year. Five lots of vaccine consisting of 304 liters were prepared. A total of 11,085 eggs were processed into 6,342 g tissue to prepare 2,195 rolling cultures of CEC. All testing has been satisfactory thus far.

II. ProcessingA. Tissue Culture

Lots C-84-2, -3, -4, -5 and -6 were produced this year from 11,085 9-day embryonated eggs. A total of 6,342 g tissue (0.57 g/egg) was processed into 2,195 rolling cultures for vaccine production.

B. Vaccine Preparation

The status of preparation and testing the five lots of vaccine is summarized in Table 11. All testing has been satisfactory to date. One lot will be prepared this next year to satisfy the vaccine requirement.

C. Vaccine Shipments

One hundred (100) vials of VEE vaccine Lot C84-1A (MNLBR 109) were shipped to USAMRIID.

III. Conclusion

The preparation of inactivated vaccine has proceeded on schedule.

Table 11

Preparation and Testing of Inactivated, Dried VEE Vaccine C-84  
(TSI-GSD 205)

Item	C-84-2	C-84-3	C-84-4	C-84-5	C-84-6
No. infected cultures (850 cm <sup>2</sup> )	377	383	400	457	465
Volume harvested (liters)	54.96	59.8	54.9	70	65
Bulk sterility:					
Control fluid	S*	S	S	S	S
Pre-filter virus	S	S	S	S	S
Post-filter virus	S	S	S	S	S
PPLO:					
Control fluid	S	S	S	S	
Pre-filter virus	S	S	S	S	
TB (Lowenstein- Jensen):					
Control fluid	S	S	S	S	S
Pre-filter virus	S	S	S	S	S
Infectivity (neg log <sub>10</sub> ):					
Pre-filter virus	ND*/9.9/9.04	ND/9.86/9.40	ND/9.58/8.49	ND/9.50/9.33	ND/9.30
Post filter virus	9.75/10.61/8.96	10.47/10.09/9.38	9.7/9.69/8.91	9.90/9.50/9.45	/9.90
Inactivating virus-					
0 time	9.75/9.90/ND	9.72/8.86/ND	8.58/9.0/ND	9.2/9.05**	/7.90
6 hr	<1/1.1/ND	<1/1.4/ND	2.72/2.5/ND	1.9/3.3**	/2.1
24 hr	<Und/<Und/ND	<Und/<Und/ND	<Und/<Und/ND	<Und/<Und	/<Und
72 hr	<Und/<Und/ND	<Und/<Und/ND	<Und/<Und/ND	<Und/<Und	/<Und
Bulk vaccine residual virus (SM)	None detected	None detected	None detected	None detected	

continued -----

Table 11 ----- CONTINUED

Preparation and Testing of Inactivated, Dried VEE Vaccine C-84  
(TSI-GSD 205)

Item	C-84-2	C-84-3	C-84-4	C-84-5	C-84-6
Vaccine Run 1:					
Bulk volume (liters)	11.42	12	11.65	12.4	9.60
Residual formalin(%)	0.005	0.005	0.005	0.015**	0.003
Bulk sterility	S	S	S	S	S
F.C. residual moisture(%)	0.26	0.25	0.24	0.29	
F.C. general safety	S	S	S	S	S
F.C. sterility	S	S	S	S	S
pH-reconstituted	6.85	6.7	6.34	6.9	6.65
Vaccine Run 2:					
Bulk volume (liters)	20	23.95	21.15	22.5	17.48
Residual formalin(%)	0.006	0.006	0.005	0.014	0.003
Bulk sterility	S	S	S	S	S
F.C. residual moisture(%)	0.35	0.20	0.23	0.23	
F.C. general safety	S	S	S	S	S
F.C. sterility	S	S	S	S	S
pH-reconstituted	6.9	6.73	6.72	7.14	6.75
Vaccine Run 3:					
Bulk volume (liters)	11.37	23.7	21.83	22.5	17.90
Residual formalin(%)	0.006	0.005	0.006	0.006	0.003
Bulk sterility	S	S	S	S	S
F.C. residual moisture(%)	0.28	0.18	0.21	0.22	
F.C. general safety	S	S	S	S	S
F.C. sterility	S	S	S	S	S
pH-reconstituted	6.9	6.7	6.7	7.03	6.90
Vaccine Run 4:					
Bulk volume (liters)				12.16	19.30
Residual formalin(%)				0.008	0.003
Bulk sterility				S	
F.C. residual moisture(%)				0.20	
F.C. general safety				S	
F.C. sterility				S	
pH-reconstituted				7.13	7.11

1] SMICLD50/TCD50-CEC/Plaque-CEC per 0.5 ml

\*S = Test Satisfactory. ND = Not Done.

\*\*Retested.

Storage Stability of Live, Attenuated  
TC-83 VEE Vaccine

I. Introduction

Storage stability of TC-83, VEE vaccine was evaluated this year.

II. Process Studies

Table 12 summarizes the CEC infectivities of liquid Lots 4 and 1-71 after 15 and 9 years respectively at  $-75^{\circ}\text{C}$ . Also Lots 4, 4-2 (10 dose) and 4-3 (10 dose) freeze-dried vaccines after 15, 8 and 8 years at  $-20^{\circ}\text{C}$ . There appears to be no detectable drop after storage under these conditions.

III. Conclusion

TC-83 vaccine is stable in the liquid state at  $-75^{\circ}\text{C}$  for at least 15 years and at least 8 years in the freeze-dried state at  $-20^{\circ}\text{C}$ .

Table 12

Storage of Liquid and Dried TC-83 VEE Vaccine  
at -20°C or -75°C

Lot No.	Physical State	Storage Temp.	0	Time in Storage		
				8 yr. (TCD <sub>50</sub> /PFU-Neg Log <sub>10</sub> )	9 yr.	15 yr.
4	Liquid	-75°C	9.6/ND			9.3/8.7
1-71	Liquid	-75°C	9.8/8.2		9.5/8.9	
4	Freeze-dried	-20°C	6.3/ND			6.0/4.0
4-2	Freeze-dried	-20°C	5.2/3.6	6.0/4.6		
4-3	Freeze-dried	-20°C	5.0/ND	6.5/5.0		

Chikungunya HA Antigen (Strain 168)  
(Sucrose-Acetone Extracted Suckling Mouse Brain)

I. Introduction

Chikungunya Strain 168 was passed twice in suckling mice and brain tissue was extracted with sucrose-acetone. Six batches were extracted and Lot 1 of the series was shipped to USAMRIID.

II. Processing

Chikungunya Strain 168 was passed at  $10^{-4}$  prior to one passage at  $10^{-3}$  to collect infected suckling mouse brains for extraction. A total of 61.2 g brain tissue was harvested from 307 7-8 day old suckling mice. Six extractions were used to process the tissue. Lot 1 was processed in a routine fashion, being diluted 1:4 in borate saline containing 0.4% HSA after inactivating with 0.3% beta-propiolactone (BPL). The remaining five lots contain no HSA, and with the exception of Lot 3, were inactivated with 0.2% BPL as shown in Table 13. These five lots were diluted 1:2 during inactivation.

Lot 1 was distributed, 25 vials of one ml each and one vial of 29.5 ml (1:1024), and shipped to USAMRIID after passing Vero and suckling mouse safety tests. The remaining five extractions were pooled and frozen at  $-70^{\circ}\text{C}$  after passing the Vero safety test.

Table 13  
Chikungunya HA Antigen  
(Sucrose-Acetone Extracted Suckling Mouse Brain)

Extraction No.	Volume (ml)	HA Titer	HSA Present (%)	Inactivating BPL Conc. (%)
1*	64	1:1024	0.4	0.3
2	30	1:2048	0	0.2
3	30	1:2048	0	0.3
4	30	1:1024	0	0.2
5	28	1:2048	0	0.2
6	38	1:2048	0	0.2

\*Shipped to USAMRIID. The remainder was pooled and frozen at  $-70^{\circ}\text{C}$ .

Venezuelan Equine Encephalomyelitis (VEE)  
HA Antigen  
(Trinidad Strain)  
(Sucrose-Acetone Extracted Suckling Mouse Brain)

I. Introduction

One batch of infected suckling mouse brains was harvested when 10% mortality occurred and the tissue was processed in seven extractions. A pool at a titer of 1:2560 was prepared and frozen at  $-70^{\circ}\text{C}$ .

II. Processing

A  $10^{-3}$  dilution of VEE, Trinidad Strain (GP1E14) was inoculated ic into suckling mice and tissue was harvested when 10% mortality occurred. The tissue was processed in seven extractions, Table 14, pooled after passing the Vero safety test and frozen at  $-70^{\circ}\text{C}$ . A dilution of 1:160 yields 8 units in the HAI test and 1:240 yields 4 units. The pool titers 1:2560.

Table 14

VEE (Trinidad) HA Antigen\*  
(Sucrose-Acetone Extracted Suckling Mouse Brain)

Extraction No.	Volume (ml)	Titer (reciprocal)
1	16	2560
2	33	2560
3	31	1024
4	35	2048
5	25	1024
6	33	>2048
7	23	2048

\*Pool titers 1:2560 -- stored at -70°C.

Eastern Equine Encephalitis (EEE) HA Antigen  
(PE6 Strain)  
(Sucrose-Acetone Extracted Suckling Mouse Brain)

I. Introduction

One batch of infected suckling mouse brains was harvested and processed with seven extractions. A pool was made and frozen at  $-70^{\circ}\text{C}$ .

II. Processing

Suckling mice were infected with a  $10^{-3}$  dilution of EEE, PE6 (Production Seed) and brain tissue was harvested when 10% mortality occurred. Seven extractions, Table 15, were made and, following a Vero safety test, a pool was prepared and frozen at  $-70^{\circ}\text{C}$ . A dilution of 1:400 yields 8 units in the HAI test and 1:600 gives 4 units.

Table 15

EEE (PE6 Strain) HA Antigen\*  
(Sucrose-Acetone Extracted Suckling Mouse Brain)

Extraction No.	Volume (ml)	HA Titer
1	25	2560
2	30	2560
3	28	5120
4	27	2560
5	30	2560
6	30	2560
7	22	2560

\*Pooled and frozen at  $-70^{\circ}\text{C}$ .

Rift Valley Fever (RVF) HA Antigen  
(Entebbe Strain)  
(Sucrose-Acetone Extracted Suckling Mouse Liver)

I. Introduction

The preparation of antigen with a titer of 1:1024 as requested (Ref. SGRD-UIV-1 3/1/79 and 7/17/79) was completed this year.

A total of 77 extractions was made and 15 batches of infected suckling mouse livers were harvested. Lot 1-80, 1800 vials, was shipped to USAMRIID. The remaining antigen is held in -20°C storage, Lot 2-80 and at -70°C, Lot 3-80 frozen liquid.

Two batches of antigen were prepared without HSA present for use in preparing a hybridoma. Batch 37-80-3 (1:4096) was shipped to USAMRIID.

II. Processing of Antigen Pools

A. Lot 1-80

As shown in Table 16, 2109 vials of Lot 1-80 were processed from 2.5 liters of pooled antigen. Of these, 227 vials had no vacuum and 1800 were shipped to USAMRIID. A dilution of 1:40 yielded 8 units for the HAI test and a dilution of 1:4 was optimal in the CF test. The Vero and suckling mouse safety tests were satisfactory.

B. Lot 2-80

A total of 2591 vials of Lot 2-80 were prepared from 3.5 liters of pooled antigen (Table 16). A dilution of 1:40 yielded 4-8 units in the HAI test and the Vero and suckling mouse safety tests were satisfactory. The vials were labeled, packed and stored at -20°C.

C. Lot 3-80

A pool of 535 ml from 14 extractions was prepared at 1:1024. It is currently held at -70°C in the frozen state. The Vero safety test was satisfactory for the pool.

D. Antigen for Use in Preparing Hybridoma

Two batches, 36-80-2 and 37-80-3, at a titer of 1:4096 were extracted and inactivated with BPL in the absence of HSA. Both passed Vero safety tests and Batch 37-80-3 passed the suckling mouse safety test prior to being shipped to USAMRIID (16 ml) for use in preparing a hybridoma. Batch 36-80-2 is held at -70° C.

### III. Conclusion

The preparation of requested antigen was completed during 1980.

Table 16

RVF (Entebbe Strain) HA Antigen Preparation  
(Sucrose-Acetone Extracted Suckling Mouse Liver-BPL Inactivated)

Lot No.	Date Freeze-Dried	No. Batches in Pool	Pool Volume (liters)	HA Titer	Moisture Content	No. Bottles
1-80	3/10/80	26 <sup>1</sup>	2.5	1:1024	0.23%	2109
2-80	8/5/80	46	3.5	1:512-1024	0.3%	2591
3-80	ND <sup>2</sup>	14	0.5	1:1024	-	480 <sup>3</sup>

32

<sup>1</sup> 19 extractions done in 1979.

<sup>2</sup> ND = Not Done, pool frozen at -70°C.

<sup>3</sup> If processed.

Eastern Equine Encephalitis (EEE) Vaccine  
Storage Stability Potency Testing

I. Introduction

Two runs of EEE vaccine Lot 1 (NDBR 104) stored at  $-20^{\circ}\text{C}$  for ten years were potency tested.

II. Potency Test Results

Potency tests performed on two runs of EEE vaccine Lot 1 (NDBR 104) were completed and the results are shown in Table 17.

These tests show that the two runs of Lot 1 have identical potency values which have not decreased during ten years storage at  $-20^{\circ}\text{C}$ .

Table 17

EEE Vaccine (NDBR 104) Storage Stability Test  
Results

Lot 1 Run*	1974	1976 (ED <sub>50</sub> )	1980
1	0.006	0.013	0.007
2	0.007	0.007	0.007

\*The one lot of vaccine was freeze-dried in two runs.

Q Fever Vaccine  
Storage Stability Potency Testing

I. Introduction

Two lots of Q fever vaccine (NDBR 105) were put on potency test.

II. Potency Test

Lots 2 and 4 Phase 1 Q Fever Vaccine (NDBR 105) prepared ten years ago were placed on potency test December 1980. During this period prevaccination sera were obtained from guinea pigs followed by a two dose regimen of vaccine at a one week interval subsequently obtaining a post vaccination sample of sera two weeks after completion of the vaccination series.

Serology by complement fixation testing should be completed during January 1981.

## Rocky Mountain Spotted Fever (RMSF) Vaccine

### I. Introduction

Potency tests were performed on Rocky Mountain Spotted Fever Vaccine (MNLBR 108) Lots 1, 2 and 3 and USAMRIID Lot 1 incorporating changes made by USAMRIID.

The above four lots of RMSF vaccines were again potency tested with protocols further modified by USAMRIID.

RMSF vaccine from Lots 2 and 3 were sent to USAMRIID.

### II. Potency Test Results

A detailed summary of potency test results from Lots 1, 2 and 3 (MNLBR 108) and Lot 1 (USAMRIID) was sent to USAMRIID April 3, 1980. These data showed that MNLBR 108 Lot 1 was the poorest vaccine in terms of total deaths, death of animals given undilute vaccine and febrile responses. The other three vaccines appeared to be approximately the same.

Following further modification of protocols from USAMRIID, a group of guinea pigs, to serve as immune controls, were treated with Acromycin, following infection with the Sawtooth strain of RMSF, until temperatures returned to normal. The potency testing, which followed, was repeated on the four lots listed above and a report containing test results was sent to USAMRIID November 17, 1980. This report indicates MNLBR 108 Lot 3 to be the best vaccine, with USAMRIID Lot 1 second in potency, followed by MNLBR 108 Lot 2. MNLBR 108 Lot 1 was the poorest vaccine tested, which was borne out by the previous potency test.

### III. Vaccine Shipment

A shipment of 15 x 2 dose vials of Lot 2 and 15 x 2 dose vials of RMSF Vaccine, Inactivated, Dried (MNLBR 108) was made, via Federal Express, to USAMRIID.

## Immunization of Employees

### I. Introduction

Plaque reduction neutralization tests were performed on pre and post vaccination sera from five employees receiving inactivated, VEE vaccine, C-84-1. Three subjects developed antibody titers greater than the required 1:80 and two did not.

### II. Results

The plaque reduction neutralization tests were performed in accordance with protocols in use at USAMRIID.

The data in Table 18 show that three employees had significant antibody titers against VEE and two employees did not.

Table 18

Neutralizing Antibody Titers in Sera Following  
Administration of VEE Vaccine, Inactivated (C-84-1)

Vaccinee	Antibody Titer*	
	Pre-immunization Serum	Post Immunization Serum
	(reciprocal serum dilution)	
HM	<1:10	1:14
RC	1:19	>1:640
AA	1:24	>1:640
VG	<1:10	1:16
RV	<1:40	1:446

\*Plaque reduction neutralization test, 80% end point.